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Melatonin and a Spin-Trap Compound Block Radiofrequency Electromagnetic Radiation-Induced DNA Strand Breaks in Rat Brain Cells

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Effects of in vivo microwave exposure on DNA strand breaks, a form of DNA damage, were investigated in rat brain cells. In previous research, we have found that acute (2 hours) exposure to pulsed (2 μsec pulses, 500 pps) 2450-MHz radiofrequency electromagnetic radiation (RFR) (power density 2 mW/cm^2, average whole body specific absorption rate 1.2 W/kg) caused an increase in DNA single- and double-strand breaks in brain cells of the rat when assayed 4 hours post exposure using a microgel electrophoresis assay. In the present study, we found that treatment of rats immediately before and after RFR exposure with either melatonin (1 mg/kg/injection, SC) or the spin-trap compound N-tert-butyl-α-phenylnitrone (PBN) (100 mg/kg/injection, IP) blocks this effect of RFR. Since both melatonin and PBN are efficient free radical scavengers, it is hypothesized that free radicals are involved in RFR-induced DNA damage in the brain cells of rats. Since cumulated DNA strand breaks in brain cells can lead to neurodegenerative diseases and cancer and an excess of free radicals in cells has been suggested to be the cause of various human diseases, data from this study could have important implications for the health effects of RFR exposure. *Bioelectromagnetics 18:446–454, 1997.*

Key words: radiofrequency electromagnetic radiation (RER); brain cells; DNA single- and double-strand breaks; melatonin; N-tert-butyl-α-phenylnitrone (PNB); free radicals

INTRODUCTION

Recently, we reported an increase in DNA single- and double-strand breaks in the brain cells of rats exposed for 2 hours to pulsed 2450-MHz radiofrequency electromagnetic radiation (RFR) at averaged whole body specific absorption rates (SAR) of 0.6 and 1.2 W/kg [Lai and Singh, 1995, 1996]. In these experiments, DNA strand breaks were assayed 4 hours post exposure.

The mechanism by which RFR causes this effect is not known. In the present study, we investigated whether free radicals play a role. Rats were treated with the free radical scavengers melatonin and N-tert-butyl-α-phenylnitrone (PBN) to investigate whether they can block RFR-induced DNA single- and double-strand breaks in brain cells. Melatonin has been reported to be a free radical scavenger [Reiter et al., 1995]. It has been shown to inhibit DNA-adduct formation induced by the carcinogen safrole in vivo [Tan et al., 1993] and to protect lymphocytes from radiation-induced chromosome damage in vitro [Vijayalaxmi, 1995]. In addition, an advantage of using melatonin in this study is that it can readily pass through the blood-brain barrier and cell and nuclear membranes [Costa et al, 1995; Menendez-Pelaez and Reiter, 1993; Menendez-Pelaez et al, 1993]. PBN has been shown to protect cells from free radical-induced apoptosis [Slater et al., 1995]. In particular, various studies have reported that PBN can reverse free radical-related damage to the nervous system. For example, it has been shown to

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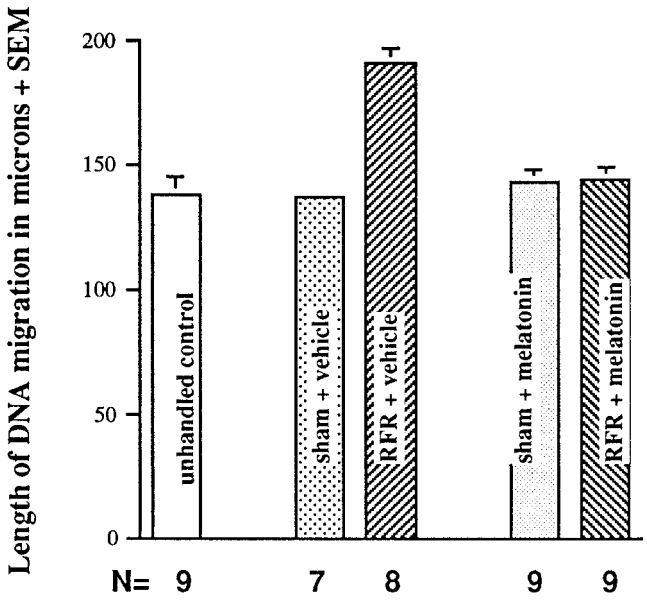

Fig. 1. Effect of treatment with melatonin on RFR-induced increase in DNA single-strand breaks in rat brain cells. Data was analyzed using the one-way ANOVA, which showed a significant treatment effect ($F[4,37] = 16.59$, $P < .001$).

reverse age-related changes in protein chemistry in the brain and deterioration in spatial memory functions in the rat [Carney and Floyd, 1991; Carney et al., 1991]. It can reverse ischemia-induced free radical injury in the brain [Oliver et al., 1990], inhibit free radical release after experimental brain concussion [Sen et al., 1994], and reduce infarct size in the brain following transient middle cerebral artery occlusion [Zhao et al., 1994].

METHODS AND PROCEDURES

Animals

Male Sprague-Dawley rats (250-300 g) purchased from B & K Laboratory, Bellevue, WA, were used in this research. They were housed three to a cage in a room adjacent to the RFR exposure room for 48 hours before an experiment. The laboratory was maintained on a 12-hour light-dark cycle (light on 6:00–18:00 h) and at an ambient temperature of 22 °C and a relative humidity of 65%. Animals were given food and water ad libitum.

RFR Exposure System and Exposure Conditions

The cylindrical waveguide system developed by Guy et al. [1979] was used for RFR exposure. The system consists of individual cylindrical waveguide tubes connected through a power divider network to a single RFR power source. Each tube consists of a section of circular waveguide constructed of galvanized wire screen in which a circularly polarized TE_{11} mode field configuration is excited. The tube contains a plastic chamber that houses a rat with enough space to allow free motion. The floor of the chamber is formed of glass rods, allowing waste to fall through plastic funnels into a collection container outside the waveguide. Waveguides were calibrated and checked from time to time.

This waveguide system, using circularly polarized radiation, enables efficient coupling of radiation energy to the animal exposed. For example, a spatially averaged power density of 1 mW/cm^2 in the circular waveguide produces a whole-body SAR of 0.6 W/kg in the rat [Chou et al., 1984]. The range of power densities for exposure to a linearly polarized plane-wave associated with an SAR of 0.6 W/kg is approximately 3–6 mW/cm^2. By connecting this system to a pulsed signal source (Applied Microwave, model PG5KB), rats were irradiated with pulsed (2 μsec pulse width, 500 pulses per second) 2450-MHz radiation at a spatially averaged power density of 2 mW/cm^2, which gave an averaged whole-body SAR of 1.2 W/kg. Since each waveguide can be activated individually, an animal can be subjected to either RFR- or sham-exposure in a waveguide. Both RFR- and sham-exposed animals were included in each exposure session.

In the experiment, animals were injected with melatonin (Sigma Chemical Co., St. Louis, MO; 1 mg/kg/injection, SC, dissolved in a concentration of 1 mg/ml in 1% ethanol-saline solution) or an equal volume of its vehicle, or with N-tert-butyl-α-phenylnitrone (PBN) (Sigma Chemical Co., St. Louis, MO; 100 mg/kg/injection, IP, dissolved at 25 mg/ml in physiological saline) or an equal volume of its vehicle. Injections were given immediately before and after exposure. The drug dosages used were based on previous studies showing efficient free radical scavenging effects, especially in the brain [Carney et al., 1991; Chen et al., 1994; Kothari et al., 1995; Lafon-Cazal et al., 1993a,b; Melchiorri et al., 1995; Tan et al., 1993; Zhao et al., 1994]. Melatonin and PBN solutions were prepared immediately before injection, and exposure to light and air were kept at a minimum. Since the drugs have a short half-life (0.5–2 hours) in the blood, the experimental schedule involved two hours of exposure and four hours of post-exposure waiting, and the exact time when DNA strand breaks occurred was not known, we decided to inject the animals twice: before and after exposure.

Therefore, there were four treatment groups for each drug (melatonin and PBN)-treatment experiment: RFR/drug; RFR/vehicle; sham/drug; and sham/vehicle.

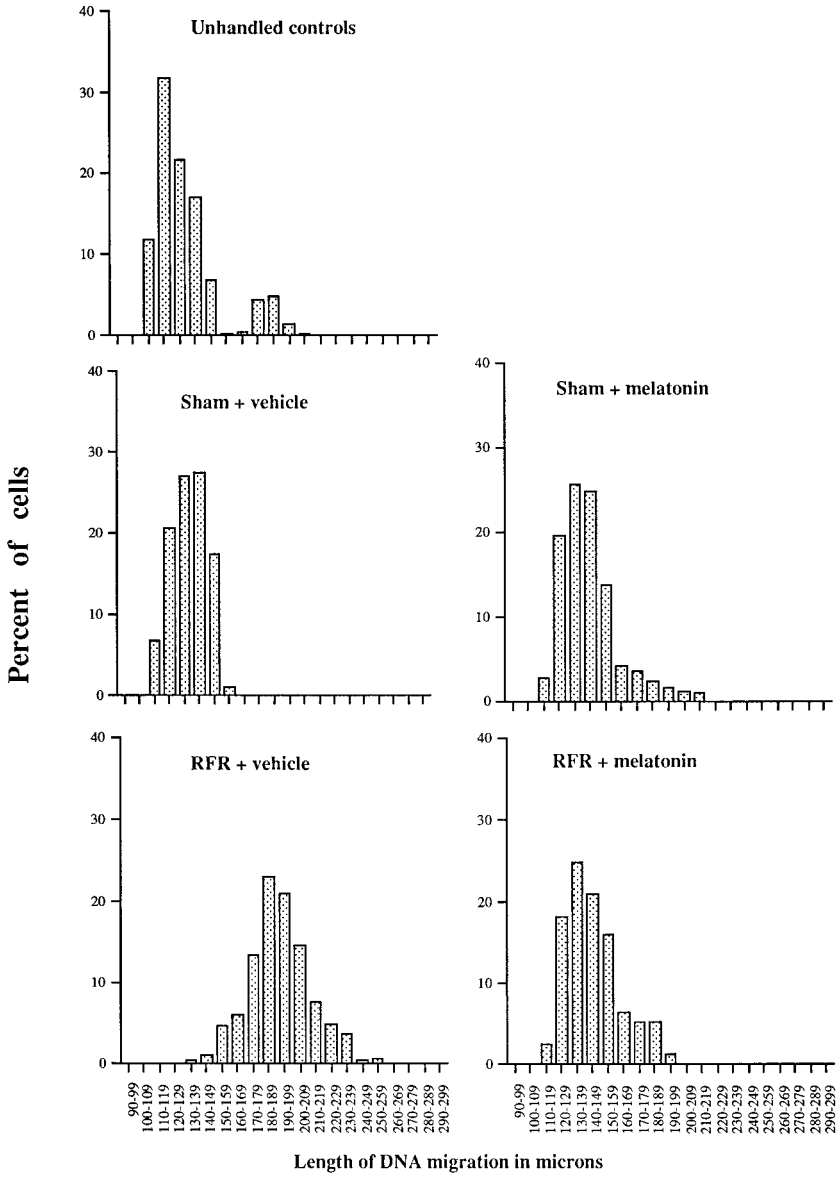

Fig. 2. Percent distribution of cells as a function of DNA migration length of the five groups of animals shown in Fig. 1.

In addition, a group of unhandled animals was included in each experiment. These animals were housed in their home cage for the entire period of the experiment, and DNA strand breaks were assayed in their brains without experimental treatment and handling. These animals controlled for the possible effect of experimental procedures on DNA strand breaks in brain cells.

The animals were returned to their home cages after exposure. Four hours later, each rat was placed for 60 seconds in a closed foam box containing dry ice (a cardboard was put on top of the dry ice to prevent its direct contact with the animal) and then decapitated with a small animal guillotine. Dry ice was used in the euthanasia because its use minimizes red blood cell contamination of tissue samples which could affect DNA strand break measurements. All procedures from this step onward were done in minimum indirect light. Brains were immediately dissected out from the skull for assay of DNA strand breaks. Dissection of a brain took approximately 30 seconds.

All experiments were run blind. The on/off conditions of the waveguides were determined by an experimenter before an experiment. Two other experimenters, who did the animal exposure/brain dissection and

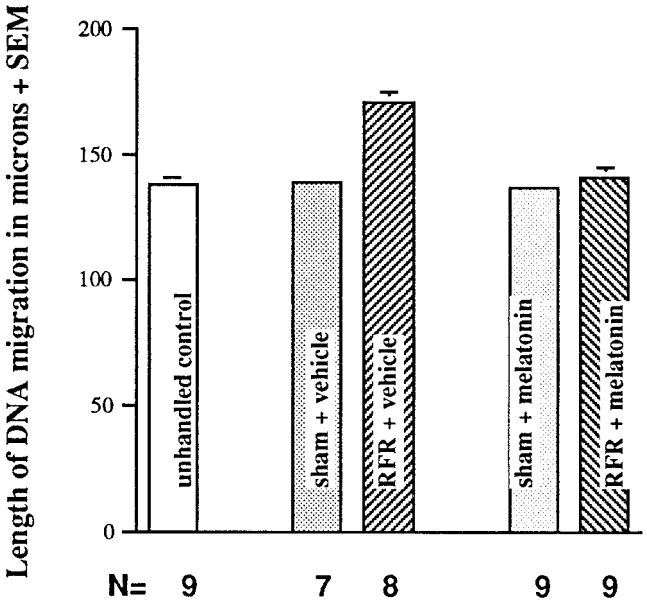

Fig. 3. Effect of treatment with melatonin on RFR-induced increase in DNA double-strand breaks in rat brain cells. One-way ANOVA of the data showed a significant treatment effect ($F[4,37] = 19.02$, $P < .001$).

DNA strand-break assay, respectively, did not know the treatment conditions (RFR or sham exposure) of the rats.

Assay Methods for DNA Strand Breaks

The microgel electrophoresis assay for DNA single- and double-strand breaks in rat brain cells was carried out as described previously in Lai and Singh [1996]. All chemicals used in the assay were purchased from Sigma Chemical Company (St. Louis, MO) unless otherwise noted. Immediately after dissection, a brain was immersed in ice-cold phosphate-buffered saline (PBS) (NaCl, 8.01 g; KCl, 0.20 g; Na_2HPO_4, 1.15 g; KH_2PO_4, 0.20 g, per liter, pH 7.4) containing 200 μM of N-t-butyl-α-phenylnitrone. The tissue was quickly washed four times with the PBS to remove most of the red blood cells. A pair of sharp scissors was used to mince (approximately 200 cuts) the tissue in a 50-ml polypropylene centrifuge tube containing 5 ml of ice-cold PBS to obtain pieces of approximately 1 mm^3. Four more washings with cold PBS removed most of the remaining red blood cells. Finally, in 5 ml of PBS, tissue pieces were dispersed into single-cell suspensions using a P-5000 Pipetman. This cell suspension consisted of different types of brain cells. Ten microliters of this cell suspension were mixed with 0.2 ml of 0.5% agarose (high-resolution 3:1 agarose; Amresco, Solon, OH) maintained at 37 °C, and 30 μ1 of this mixture was pipetted onto a fully frosted slide (Erie Scientific Co., Portsmouth, NH) and immediately covered with a 24 × 50 mm square #1 coverglass (Corning Glass Works, Corning, NY) to make a microgel on the slide. Slides were put in an ice-cold steel tray on ice for 1 min to allow the agarose to gel. The coverglass was removed and 200 μl of agarose solution was layered as before. Slides were then immersed in an ice-cold lysing solution (2.5 M NaCl, 1% sodium N-lauroyl sacosinate, 100 mM disodium EDTA, 10 mM Tris base, pH 10) containing 1% Triton X-100.

To measure single strand DNA breaks, after lysing overnight at 4 °C, slides were treated with DNAase-free proteinase K (Boehringer Mannheim Corp., Indianapolis, IN) in the lysing solution for 2 hours at 37 °C. They were then put on the horizontal slab of an electrophoretic assembly (Hoefer Scientific, San Francisco, CA) modified so that both ends of each electrode were connected to the power supply. One liter of an electrophoresis buffer (300 mM NaOH, 0.1% of 8-hydroxyquinoline, 2% dimethyl sulfoxide, and 10 mM tetra-sodium EDTA, pH 13) was gently poured into the assembly to cover the slides to a height of 6.5 mm above their surface. After allowing 20 min for DNA unwinding, electrophoresis was started (0.4 volt/cm, approximately 250 mA, for 60 min) and the buffer was recirculated.

At the end of the electrophoresis, electrophoretic buffer above the slides was gently removed. Slides were then removed from the electrophoresis apparatus and immersed in neutralization buffer (0.4 M Tris at pH 7.4) in a Coplin jar (two slides per jar) for 10 min. After two more similar steps of neutralization, the slides were dehydrated in absolute ethanol in a Coplin jar for 30 min and then dried.

For double-strand breaks, microgel preparation and cell lysis were done as mentioned above. Slides were then treated with ribonuclease A (Boehringer Mannheim Corp., Indianapolis, IN) (10 μg/ml in the lysing solution) for 2 hours and then with proteinase K (1 mg/ml in the lysing solution) for 2 hours at 37 °C. They were then placed for 20 min in an electrophoretic buffer (100 mM Tris, 300 mM sodium acetate and acetic acid at pH 9.0), and then electrophoresed for 1 hour at 0.4 volt/cm (approximately 100 mA). The slides were treated with 300 mM NaOH for 10 min and neutralized as before with 0.4 M Tris (pH 7.4). Slides were then dehydrated in absolute ethanol for 30 min and dried.

Staining and DNA migration measurement procedures were similar for both single- and double-strand breaks. One slide at a time was taken out and stained with 50 μl of 1 μM solution of YOYO-1 (stock, 1 mM in DMSO from Molecular Probes, Eugene, OR) and then covered with a 24 × 50-mm coverglass. Slides were examined and analyzed with a Reichert vertical fluores-

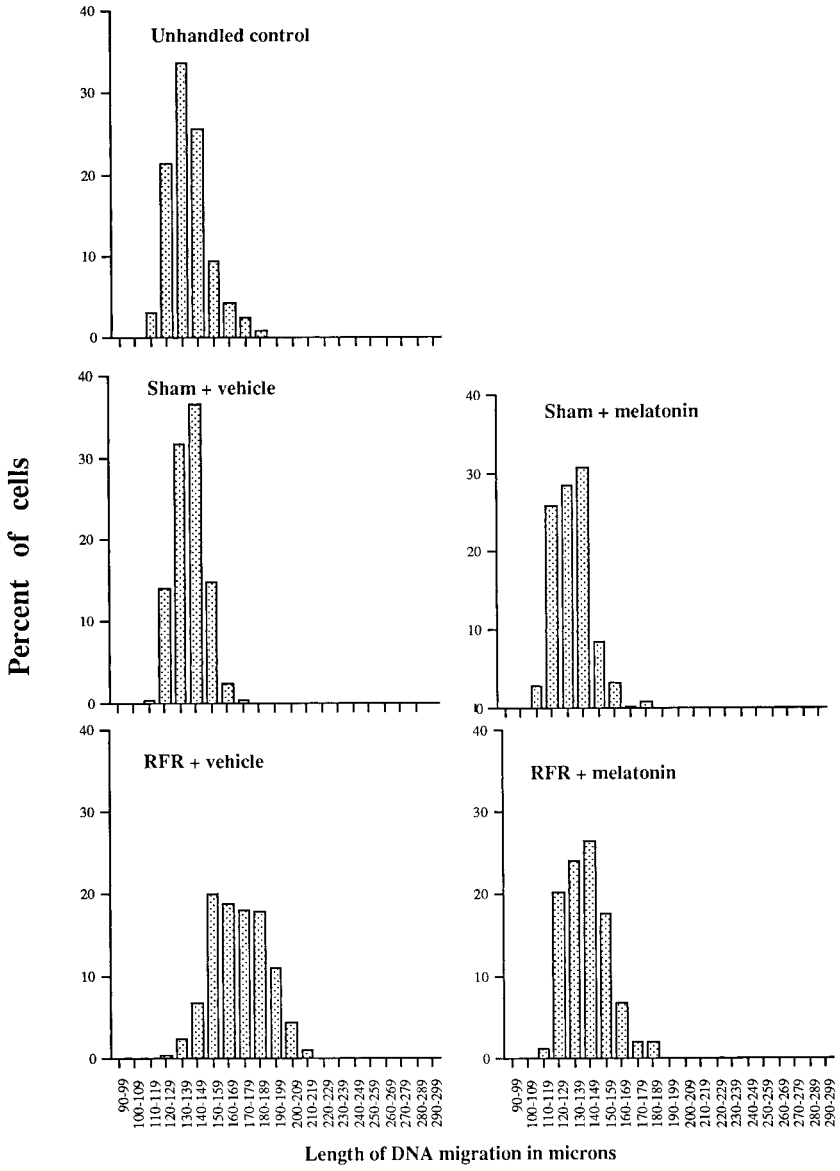

Fig. 4. Percent distribution of cells as a function of DNA migration length of the five groups of animals shown in Fig. 3.

cent microscope (model 2071) equipped with a filter combination for fluorescence isothyocynate (excitation at 490 nm, emission filter at 515 nm, and dichromic filter at 500 nm). We measured the length of DNA migration (in microns) from the beginning of the nuclear area to the last 3 pixels of DNA perpendicular to the direction of migration at the leading edge. The migration length is used as the index of DNA strand breaks. In the present assay procedure, precipitation with ethanol enabled detection of smaller DNA fragments and increased the sensitivity and resolution of the assay. With this treatment, a significantly higher DNA migration length was detected. Without ethanol precipitation, the migration lengths of DNA from brain cells of a sham-exposed animal would be 40–50 microns.

Two slides were prepared from the brain sample of each animal: one for assay of single-strand DNA breaks, and the other for double-strand breaks. Fifty cells were randomly chosen and scored from each slide. However, cells that showed extensive damage, with DNA totally migrated out from the nuclear region, were not included in the measurement. These highly damaged cells probably resulted from the tissue and cell processing procedures. They occurred equally in RFR-

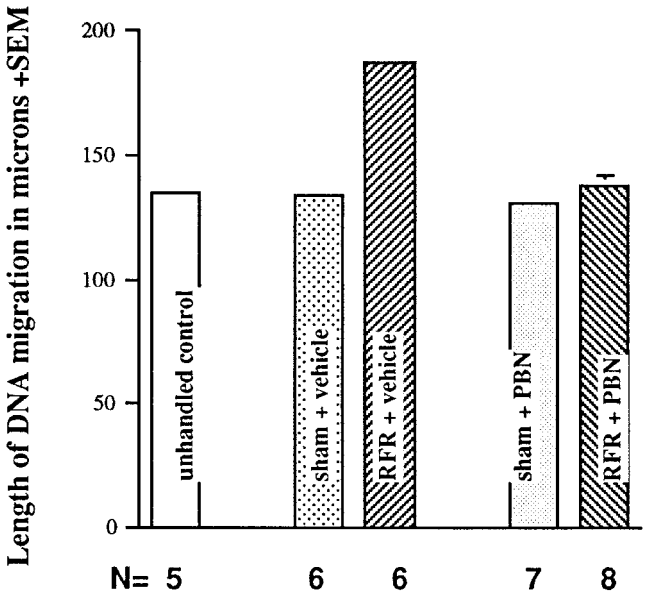

Fig. 5. Effect of treatment with PBN on RFR-induced increase in DNA single-strand breaks in rat brain cells. Data was analyzed using the one-way ANOVA, which showed a significant treatment effect (F[4,27]= 75.5, $P < .001$).

exposed, sham-exposed, and unhandled samples. Therefore, from each animal, 50 cells each were scored for single- and double- strand DNA breaks.

Data Analysis

The average length of DNA migration from the 50 cells, measured for single- and double-strand breaks in each rat, was used in data analysis using the one-way ANOVA. The difference between the two treatment groups was compared by the Newman-Keuls Test with a difference of $P < .05$ considered statistically significant. Percentages of cells with respect to DNA migration length (in intervals of 10 microns) were also plotted.

RESULTS

Figure 1 shows the data of melatonin treatment on RFR-induced DNA single-strand breaks in brain cells of rats. RFR significantly increased DNA single-strand breaks in brain cells ('RFR+ vehicle' vs 'sham + vehicle', $P < .01$, Newman-Keuls test), whereas treatment with melatonin completely blocked the effect of RFR (i.e., no significant effect was found between 'RFR + melatonin' and 'sham + melatonin'). It should be pointed out that melatonin by itself has no significant effect on DNA single-strand breaks, i.e., no significant difference was found between the 'sham + melatonin' and 'sham + vehicle' groups. Experimental procedures also had no significant effect on DNA single-strand breaks in brain cells of the rat (i.e., there is no significant difference between the unhandled control and 'sham + vehicle' groups). Percentage distributions of cells as a function of DNA migration length for the five treatment groups in this experiment are shown in Figure 2. Exposure to RFR caused a shift of distribution to longer lengths (i.e., to the right), and treatment with melatonin restored the distribution to a pattern similar to that of the 'sham + vehicle' animals.

A similar conclusion can be drawn from data of the study on treatment with melatonin on DNA double-strand breaks in brain cells. Melatonin treatment blocked RFR-induced increase in DNA double-strand breaks in rats brain cells. Figures 3 and 4 plot the mean migration length and percent cell versus migration length distribution, respectively.

The results of treatment with PBN on RFR-induced increase in DNA single- and double-strand breaks in rat brain cells are presented in Figures 5–8. Similar to the effect of melatonin treatment, PBN blocked the RFR-induced increases in DNA single- and double-strand breaks in rat brain cells.

DISCUSSION

Data from the present experiment confirm our previous finding [Lai and Singh, 1995, 1996] that acute RFR exposure causes an increase in DNA single- and double-strand breaks in brain cells of the rat. In addition, we have found that the effect can be blocked by treating the animals with melatonin or PBN. Since a common property of melatonin and spin-trap compounds is that they are efficient free radical scavengers [Carney and Floyd, 1991; Carney et al., 1991; Floyd, 1991; Lafon-Cazal et al., 1993 a,b; Lai et al., 1986; Oliver et al., 1990; Reiter et al., 1995; Sen et al., 1994; Zhao et al., 1994], these data suggest that free radicals may play a role in the RFR-induced DNA single- and double-strand breaks observed in brain cells of the rat. Consistent with this hypothesis is the fact that free radicals can cause damage to DNA and other macromolecules in cells. Particularly, oxygen free radicals have been shown to cause DNA strand breaks [McCord and Fridovich, 1978]. In addition, a study has implicated free radicals as the cause of some of the biological effects observed after exposure to RFR. Phelan et al. [1992] reported that RFR can interact with melanin-containing cells and lead to changes in membrane fluidity consistent with a free radical effect.

If free radicals are involved in the RFR-induced DNA strand breaks in brain cells, results from the present study could have an important implication on the health effects of RFR exposure. Involvement of free radicals in human diseases, such as cancer and atherosclerosis, have been suggested. Free radicals also play

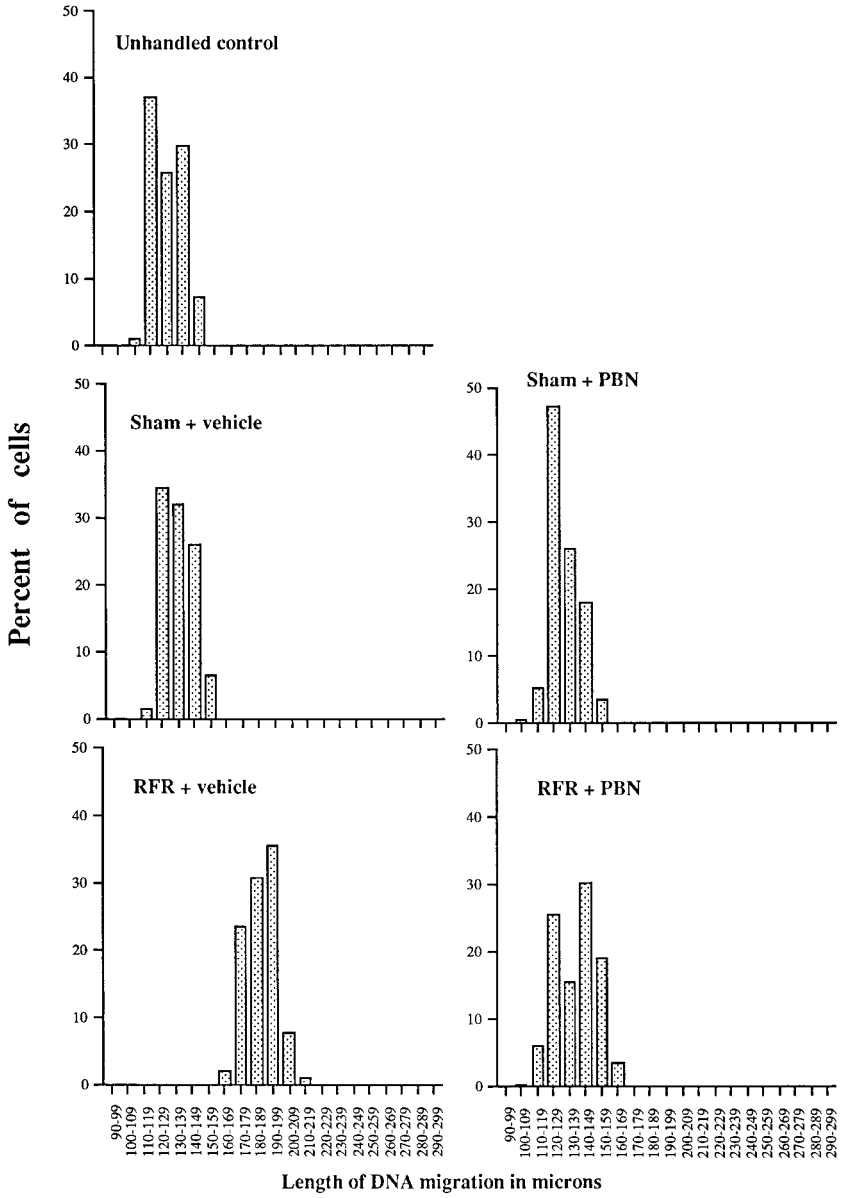

Fig. 6. Percent distribution of cells as a function of DNA migration length of the five groups of animals shown in Fig. 5.

an important role in aging processes [Reiter, 1995]. Aging has been ascribed to accumulated oxidative damage to body tissues [Forster et al., 1996; Sohal and Weindruch, 1996], and involvement of free radicals in neurodegenerative diseases, such as Alzheimer's, Huntington's, and Parkinson's, has also been suggested [Borlongan et al., 1996; Owen et al., 1996]. Furthermore, the effect of free radicals can depend on the nutritional status of an individual, e.g., availability of dietary antioxidants [Aruoma, 1994], consumption of ethanol [Kurose et al., 1996], and dietary restriction [Wachsman, 1996]. Various life conditions, such as psychological stress [Haque et al., 1994] and strenuous physical exercise [Clarkson, 1995], have been shown to increase oxidative stress and enhance the effect of free radicals in the body. Thus, one can speculate that some individuals may be more susceptible to the effects of RFR exposure.

However, it must be pointed out that both melatonin and PBN can have other actions on cells in the brain that can decrease DNA damage. Further support for our hypothesis can be obtained by studying whether other compounds with free radical scavenging properties can similarly block the effect of RFR, and by measurement of other free radical-related cellular effects, such as oxidative molecular damages in lipids, protein, and DNA.

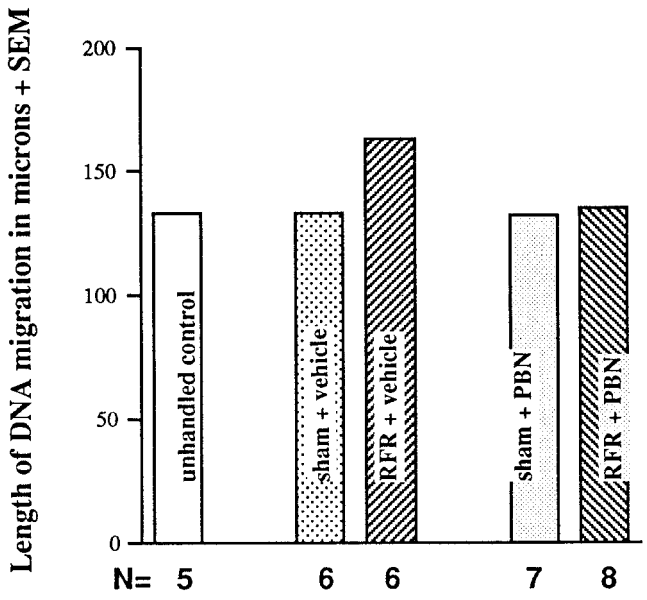

Fig. 7. Effect of treatment with PBN on RFR-induced increase in DNA double-strand breaks in rat brain cells. One-way ANOVA of the data showed a significant treatment effect ($F[4,27] = 47.83$, $P < .001$).

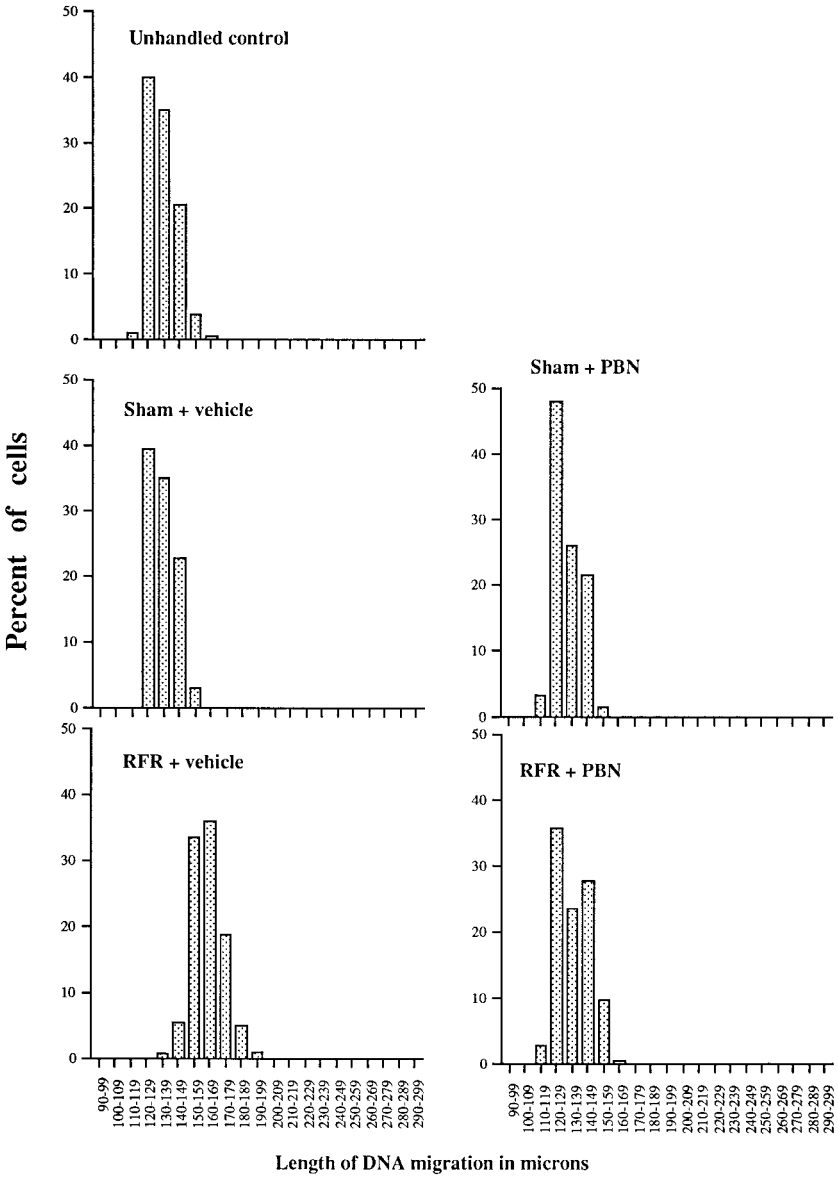

Fig. 8. Percent distribution of cells as a function of DNA migration length of the five groups of animals shown in Fig. 7.

REFERENCES

Aruoma OI (1994): Nutrition and health aspects of free radicals and antioxidants. Food Chem Toxiciol 32:671–683.

Borlongan CV, Kanning K, Poulos SG, Freeman TB, Cahill DW, Sanberg PR (1996): Free radical damage and oxidative stress in Huntington's disease. J Florida Med Assoc 83: 335–341.

Carney JM, Floyd RA (1991): Protection against oxidative damage to CNS by α-phenyl-tert-butylnitrone (PBN) and other spin-trapping agents: A novel series of nonlipid free radical scavengers. J Mol Neurosci 4:47–57.

Carney JM, Starke-Reed PE, Oliver CN, Landum RW, Cheng MS, Wu JF, Floyd RA (1991): Reversal of age-related increase in brain protein oxidation, decrease in enzyme activity, and loss in temporal and spatial memory by chronic administration of the spin-trapping compound N-tert-butyl-α-phenylnitrone. Proc Nat Acad Sci (USA) 88:3633–3636.

Chen LD, Kumar P, Reiter RJ, Tan DX, Chamber JP, Manchester LC, Poeggeler B (1994): Melatonin reduces ^{3}H-nitrendipine binding in the heart. Proc Soc Exp Biol Med 207:34–37.

Chou CK, Guy AW, Johnson RB (1984): SAR in rats exposed in 2450-MHz circularly polarized waveguide. Bioelectromagnetics 5:389–398.

Clarkson PM (1995): Antioxidants and physical performance. Crit Rev Food Sci Nutri 35:131–141.

Costa EJX, Harzar-Lopes R, Lamy-Freund MT (1995): Permeability of pure lipid bilayers to melatonin. J Pineal Res 19:123–126.

Floyd RA (1991): Oxidative damage to behavior during aging. Science 254:1597.

Forster MJ, Dubey A, Dawson KM, Stutts WA, Lal H, Sohal RS (1996): Age-related losses of cognitive function and motor skills in mice are associated with oxidative protein damage in the brain. Proc Nat Acad Sci (USA) 93:4765–4769.

Guy AW, Wallace J, McDougall JA (1979): Circular polarized 2450-MHz waveguide system for chronic exposure of small animals to microwaves. Radio Sci 14(6s):63–74.

Haque MF, Aghabeighi B, Wasil M, Hodges S, Harris M (1994) Oxygen free radicals in idiopathic facial pain. Bangladesh Med Res Council Bul 20:104–116.

Kothari A, Borges A, Kothari L (1995): Chemoprevention by melatonin and combined melatonin-tamoxifen therapy of second generation nitroso-methylurea-induced mammary tumours in rats. Eur J Cancer Prev 4:497–500.

Kurose I, Higuchi H, Kato S, Miura S, Ishii H (1996): Ethanol-induced oxidative stress in the liver. Alcohol Clin Exp Res 20(1 Suppl):77A–85A.

Lafon-Cazal M, Culcasi M, Gaven F, Pietri S, Bockaert J (1993a): Nitric oxide, superoxide and peroxynitrite: putative mediators of NMDA-induced cell death in cerebellar granule cells. Neurophysiol 32:1259–1266.

Lafon-Cazal M, Pietri S, Culcasi M, Bockaert J (1993b): NMDA-dependent superoxide production and neurotoxicity. Nature 354:535–537.

Lai EK, Crossley C, Sridhar R, Misra HP, Janzen EG, McCay PB (1986): In vivo spin trapping of free radicals generated in brain, spleen, and liver during γ radiation of mice. Arch Biochem Biophys 244:156–160.

Lai H, Singh NP (1995): Acute low-intensity microwave exposure increases DNA single-strand breaks in rat brain cells. Bioelectromagnetics 16:207–210.

Lai H, Singh NP (1996): DNA Single- and double-strand breaks in rat brain cells after acute exposure to low-level radiofrequency electromagnetic radiation. Inter J Rad Biol 69:513–521.

McCord JM, Fridovich I (1978): The biology and pathology of oxygen radicals. Ann Intern Med 89:122–127.

Melchiorri D, Reiter RJ, Attia AM, Hara M, Burgos A, Nistico G (1995): Potent protective effect of melatonin on in vivo paraquet-induced oxidative damage in rats. Life Sci 56:83–89.

Menendez-Pelaez A, Reiter RJ (1993): Distribution of melatonin in mammalian tissues: The relative importance of nuclear versus cytosolic localization. J Pineal Res 15:59–69.

Menendez-Pelaez A, Poeggeler B, Reiter RJ, Barlow-Walden LR, Pablos MI, Tan DX (1993): Nuclear localization of melatonin in different mammalian tissues: Immunocytochemical and radioimmuno assay evidence. J Cell Biochem 53:372–382.

Oliver CN, Starke-Reed PE, Stadtman ER, Liu GJ, Carney JM, Floyd RA (1990): Oxidative damage to brain proteins, loss of glutamine sythetase activity, and production of free radicals during ischemia/reperfusion-induced injury to gerbil brain. Proc Nat Acad Sci (USA)87:5144–5147.

Owen AD, Schapira AH, Jenner P, Marsden CD (1996): Oxidative stress and Parkinson's disease. Ann NY Acad Sci 786:217–223.

Phelan AM, Lange DG, Kues HA, Lutty GA (1992): Modification of membrane fluidity in melanin-containing cells by low-level microwave radiation. Bioelectromagnetics13:131–146.

Reiter R (1995): Oxidative processes and antioxidative defense mechanisms in the aging brain. FASEB J 9:526–533.

Reiter RJ, Melchiorri D, Sewerynek E, Poeggeler B, Barlow-Walden L, Chuang J, Ortiz GG, Acuna-Castroviejo D (1995): A review of the evidence supporting melatonin's role as an antioxidant. J Pineal Res 18:1–11.

Sen S, Goldman H, Morenhead M, Murphy S, Phillis JW (1994): α-phenyl-tert-butyl-nitrone inhibits free radical release in brain concussion. Free Rad Biol Med 16:685–691.

Slater AF, Nobel CS, Orrenius S (1995): The role of intracellular oxidants in apoptosis. Biochem Biophy Acta 1271:59–62.

Sohal RS, Weindruch R (1996): Oxidative stress, caloric restriction, and aging. Science 273:59–63.

Tan DX, Poeggeler B, Reiter RJ, Chen LD, Chen S, Manchester LC, Barlow-Walden LR (1993): The pineal hormone melatonin inhibits DNA-adduct formation induced by the chemical carcinogen safrole in vivo. Cancer Lett 70:65–71.

Vijayalaxmi, Reiter RJ, Meltz ML (1995): Melatonin protects human blood lymphocytes from radiation-induced chromosome damage. Mutat Res 346:23–31.

Wachsman JT (1996): The beneficial effects of dietary restriction: Reduced oxidative damage and enhanced apoptosis. Mutat Res 350:25–34.

Zhao Q, Pahlmark K, Smith M-L, Siesjo BK (1994): Delayed treatment with the spin-trap α-phenyl-N-tert-butyl nitrone (PBN) reduces infarct size following transient middle cerebral artery occlusion in rats. Acta Physiol Scand 152:349–350.